COMMITEES

National Organizing Committee
President: Dragan Gvozdić
Organizing secretary: Natalija Fratrić
Technical secretary: Branko Petrujkić

Members
Vlado Teodorović
Vera Katić
Sanja Kovačević-Aleksić
Milica Kovačevic-Filipović
Sonja Radojičić
Slobodanka Vakanjac
Horea Šamanc
Miodrag Lazarević
Ivan Jovanović
Branislav Prokić
Dragan Damnjanović
Dragan Šefer

International Scientific Committee
Prof. W. Baumgartner, PhD
Prof. O. Szenci, PhD
Ozbalt Podpecan, PhD
Borut Zemljic, PhD
Prof. J. Kos, PhD
Doc. Nikica Prvanovic, PhD
Prof. K. Doll, PhD
Prof. G. Kovác, PhD
Doc. J. Illek, PhD
J. Twardoń
Prof. Natalija Fratric, PhD

Conference Technical Organizer
BBN Congress Management doo

HEMATOLOGY AND BIOCHEMISTRY PARAMETARS IN NEONATAL CALVES WITH LOW IRON LEVELS

Danilović Luković J**, Katić M***, Lužajić T*, Blond B***, Kovačević Filipović M*

*Department of Pathophysiology, Faculty of Veterinary Medicine, University of Belgrade
**Department of Biomedical Sciences, State University of Novi Pazar
***PKB Corporation

Corresponding author: Jelena Danilović Luković, Department of Biomedical Sciences, State University of Novi Pazar, Serbia, Bulevar Oslobodjenja br. 18, e-mail: reginadelphy@yahoo.com

ABSTRACT

Serum iron (Fe) deficiency is a common problem in newborn calves in dairy industry. Iron deficiency have an influence on the immune status of calves as well as on erythropoesis and thrombopoesis. Therefore, the aim of this study was to determine Fe status in neonatal calves and to relate Fe status to basic hematological and biochemical parameters and serum fibrinogen levels. Blood was collected from 21 calves of Holstein-Friesian breed (7 female and 14 male) 12 to 24 hours after birth. Calves were divided into two groups. Group A included calves with iron concentration less than 14 μmol/L and group B calves with concentration greater than 14 μmol/L. Iron concentration, total iron binding capacity (TIBC), fibrinogen, albumin and total protein were determined using standard laboratory techniques. Leukocyte count was determined using manual and PCV using microhematocrit method. The platelet count and differential leukocyte count was obtained by blood smears examination. The results have shown that all animals at birth had iron deficiency without signs of anemia. Platelet count was higher in group B. TIBC was significantly higher and transferrin saturation was significantly lower in group A. Fibrinogen concentration, other blood elements and globulin concentration were within the previously reported values for neonatal calves. Based on these results, we supposed that concentration of iron can influence platelet count in healthy neonatal cattle. Further studies are needed to confirm these findings and to establish its physiological significance.

KEY WORDS: iron deficiency, platelets, fibrinogen, neonatal calves

INTRODUCTION

Congenital iron (Fe) deficiency is a common problem in newborn calves in dairy cows industry in Serbia (Prodanović et al., 2010; Katić, 2011). Fe deficiency and consequent neonatal anemia are considered as important predisposing factors for loss of adequate immunity and development of different pathology (Tennant et al., 1975). Fe deficiency could be the cause of enhanced platelet count in humans (Schloesser et al., 1965; Park et al., 2012). Other reported cause of increased platelet count in horses and dogs is the presence of subclinical or clinical inflammation (Sellon et al., 1997; Neel et al., 2012). Further more, inflammation in cows is characterized with increased fibrinogen concentration (Hirvonen and Pyörälä, 1998; Latimer at al., 2003).

There are no extensive literature data dialing with platelet count in newborn calves. Platelet count is an important factor that enables adequate haemostatic mechanisms in the body. Yet, it is not known if there is any relationship between iron concentration and platelet count in neonatal calves. The aim of this study was to compare platelet count, fibrinogen concentration and other hematological and biochemical parameters in calves born with low and very low iron concentration.

MATERIALS AND METHODS

The experiment was conducted on 21 Holstein-Friesian calves (7 female and 14 male). All calves were vital at the time of birth and took colostrum before blood sampling. From each calf, jugular blood samples were taken in vacutainer tubes (EDTA D-VAC, DemophoriusUK, BD Vacutainer, BD, Plymouth, UK and Na-citrate Vacuette, GreinerBio-One) 12 to 24 hours after birth. Hematological analyzes were performed 2 hours after blood sampling along with making a blood smear (Hemacolor®, Merck). Citrate and whole blood were centrifuged 15 minutes at 2500 rpm. Plasma and serum were separated and kept at -20°C until the time of analysis.

White blood cells (WBC) count was assessed using manual method. The platelet count and differential leukocyte count was obtained by blood smears examination (Harvey et al., 2001). Fibrinogen concentration was determined by Clauss method (Clauss, 1957). Standard commercial kits (Bioanalytica, Beograd, Srbija) were used for assessment of iron concentration, TIBC, total protein and albumin concentration (Spectrophotometer - RAYTO-1904C). Transferrin saturation (%) was calculated as follows = (Fe ×100%) / TIBC. Globulin concentration was calculated from total protein and albumin concentration. Packed cell volume (PCV) was obtained with microhematocrit method. After all analysis performed, using 14μmol/L as a treshold, we formed a group A (n=15) having an average of 7.2 ± 0.6 μmol/L (calves with very low Fe concentration) and a group B (n=4) having an average of $15.4 \pm 0.$ μmol/L of iron (calves with low Fe concentration) in serum.

Data analysis was performed in Excel. Data are presented using descriptive statistic parameters. Differences between the variables were assessed using the Student's t test.

RESULTS AND DISCUSSION

Our results demonstrated that 12 to 24 hours after calving all neonatal animals had iron concentration below previously refered values (Bostedt at al.,1990). Only two calves had iron concentration of 16.6μmol/L and two calves 14 μmol/L (group B), while all the other animals had lower values (Table 1). Previously, it has been suggested that 16.1 +/- 1.9 μmol/L is a mark of latent iron deficiency in calves, predicting development of iron deficiency and influencing their vitality (Bostedt at al.,1990). Presented data indicate that all calves in our study were born with iron deficiency, yet without apparent anemia (Figure 1A). Anemia is not expected at birth but when newborns are iron deficient, anemia develops during first few weeks of life, if parenteral iron supply is omitted (Bunger et al., 1980). Iron deficit in calves from our study was related to low iron concentration of their mothers during prepartal period (Katić, 2011). When other hematological and biochemical data were compared between two groups of calves, the next results were obtained: PCV and WBC values as well as differential WBC count showed no difference (Figure 1A and 1B, Table 2), while platelet count was higher within group B (Figure 1C). All hematological findings were in accordance with published average values for this category of animals (Lumsden et al., 1980). Higher platelet count in group B is in contrast with our expectations and previous results demonstrating that adult humans with iron deficiency have enhanced platelet count (Schloesser et al., 1965; Park et al., 2012). We can not exclued the possibility that platelet count in examined calves was also influenced by other variables that were not measured. It was recently evidenced that there are developmental differences in thrombopoiesis among neonatal and adult humans (Liu and Sola-Visner, 2011). As this is the first time that platelet count has been analised in iron deficient calves and that both parameters

...related to calf health, we consider that our future research should be performed on ...number of animals and measurement of different variables in aim to obtain better insight ...eonatal homeostatic mechanisms.

Table 1. Descriptive statistics data concerning iron values in two groups of calves.

groups	Fe concentration	
	n = 15 >14 µmol/L	n = 4 <14 µmol/L
mean±SE (min-max)	15.4±0.6 (14.1-16.6)	7.2±0.6 (2.7-10.5)

Table 2. Descriptive statistic data (mean±SE and min-max) concerning differential leukocyte formula in two groups of calves.

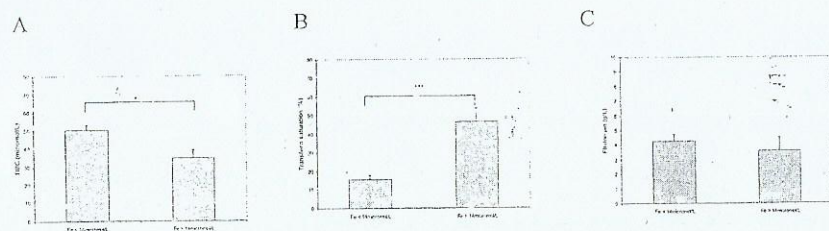| × 10⁹/L	Groups according to different Fe concentration	
	n = 15 <14 µmol/L	n = 4 >14 µmol/L
Segmented NG	7.0 ± 1.1 (1.0-14.9)	4.0 ± 1.1 (2.2-7.0)
Band NG	0.8±0.6 (0.2-2)	0.5±0.5 (0.04-1.2)
Eosinophils	0.03±0.06 (0-0.2)	0.03±0.04 (0-0.09)
Lymphocytes	1.3 ±1.2 (0.2-4.8)	1.4±1.4 (0.2-4.7)
Monocytes	0.4±0.3 (0.05-0.9)	0.3±0.2 (0.04-0.4)

A   B   C

Figure 1. TIBC, tranferrin saturation and fibrinogen concentration between two groups of calves. Data are presented as mean±standard error.
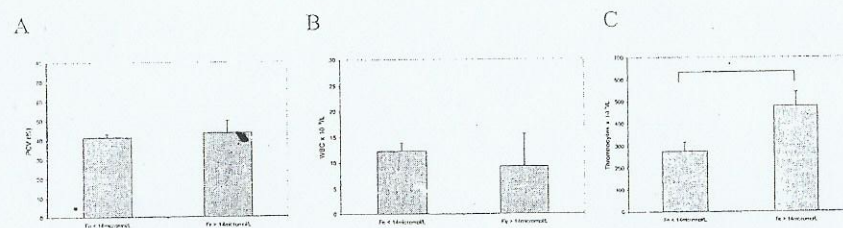
A   B   C

Figure 2. Hematological values between two groups of calves. Data are presented as mean±standard error.

Our data also demonstrated that TIBC and transferrin saturation were different among two groups (Figure 2A and 2B). These results are expected as low iron concentration stimulates transferrin synthesis (Skrzypczak *et al.*, 2009). As TIBC was higher and iron concentration lower in group A, transferrin saturation was also significanlty lower in group A. Indeed, transferrin saturation in group A was 16%, while in group B was 46%. Transferrin saturation of 46% is in upper reference limits for adult animals (Lumsden *et al.*, 1980). Calves in both groups had similar PCV, total protein and globulin concentration (Figure 2A and Figure 3A and 3C) indicating that the amount of consumed colostrum probably was similar between two groups. Concentration of albumin was lower in group of calves with higher iron concentration (Figure 3B). In both groups, all calves (except one) had fibrinogen concentration (Figure 2C) in the reference range for cattles (1 to 6 g/L - http://www.merckmanuals.com) indicating that calves did not have an acute phase response that could affect iron concentration.
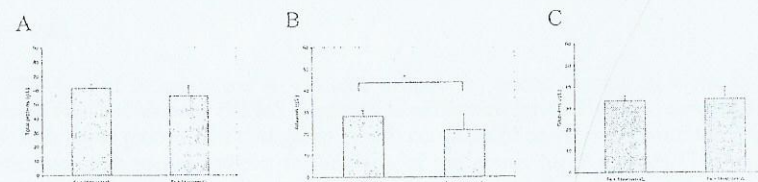
A   B   C

Figure 3. Protein values between two groups of calves. Data are presented as mean±standard error.

Concerning all data thogether, we can conclude that it is possible that iron concentration positivly affects platelet count in neonatal calves, but as multiple factors affect hematological and biochemical parameters soon after birth, larger study is needed to evaluate significance of this finding.

ACKNOWLEDGEMENTS

REFERENCES

Bostedt H, Jekel E, Schromel P, 1990, The development of iron and copper concentration in blood plasma of calves in the first days and weeks of life, equally a contribution to the larvaceous neonatal iron deficiency anemia, *Dtsch Tierarztl Wochenschr*, 97, 400-3

Bunger U, Kapha..gst P, Fiebig U, Schonfelder E, Jentsch D, Ponge J, 1980, Anaemia in male calves during rearing. 4. Relations between birth weight, duration of trial and body weight gain while the calves were fed on colostrums, and the blood picture during weaning. *Archiv fur Tierernahrung*, 30, 611-31

Clauss, A, 1957, Fast coagulation method for estimation of fibrinogen, *Acta Haematologica*, 17, 237-46

Harwey JW, 2012, Veterinary Hematology, a Diagnostic guide and Color Atlas, *Sounders Elsevier Inc*, 16-19

Hirvonen J, Pyörälä S, 1998, Acute-phase response in dairy cows with surgically-treated abdominal disorders, *Vet J*, 155, 53-61

Katić M, 2011, Parameters of iron status in newborn calves and cows of different ages on the peripartuent period, *Specialist thesis*, 60-5

Latimer K S, Mahaffey E A, Prasse K W, 2003, Veterinary Laboratory Medicine – Clinical Pathology, 4th Edition, *Blackwell Publishing*, Iowa, USA, 67-8

...ola-Visner M, 2011, Neonatal and adult megakaryopoiesis, *Curr Opin Hematol*, 18, ...-7

...sden JH, Mullen K, Rowe R, 1980, Hematology and biochemistry reference values for female Holstein cows, *Can J Comp Med*, 44, 24-31

Park M J, Park P W, Seo Y H, Kim K H, Park S H, Jeong J H, Ahn J Y, 2012, The relationship between iron parameters and platelet parameters in women with iron deficiency anemia and thrombocytosis, *Platelets*, 23, 1-4

Prodanović R, Jakić-Dimić D, Ivetić V, Savić B, Žutić M, Pavlović I, Kureljušić B, 2010, Effect of iron deficiency on health status of neonatal calves, *14th Int Symp-Feed Tehnology*, Novi Sad 19-21 October, 320-26

Schloesser L L, Kipp M A, Wenzel F J, 1965, Thrombocytosis in iron-deficiency anemia, *J Lab Clin Med*, 66, 107-14

Skrzypczak W F, Ozgo M, Lepczinsky A, Lata A, 2009, Dinamics of changes in iron concentration and total iron binding capacity in blood plasma of goat krols during their first month of life, *Archiv Tierzacht*, 52, 419-24

Tennant B, Harrold D, Reina-Guerra M, Kaneko J J, 1975, Hematology of the neonatal calf.III.Frequency of congenital iron deficiency anemia, *Cornell Vet*, 65, 543-56

Neel, J A, Snyder L, Grindem C B, 2012, Thrombocytosis: a retrospective study of 165 dogs, *Vet Clin Pathol*, 41, 216-22

Sellon D C, Levin J F, Palmer K, Millikin E, Grindem C, Covington P, 1997, Thrombocytosis in 24 horses (1989-1994), *J Vet Intern Med*, 11, 24-9.

OZONE THERAPY OF RETAINED PLACENTA IN HOLSTEIN COWS

Đuričić D[1], Ablondi M[2], Samardžija M[3], Tomica D[4], Herceg Ž[4], Dobranić T.[3]

[1] Veterinary practice Đurđevac d.o.o., Croatia, [2] Veterinary practice Parma, Italy, [3] Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia, [4] KBC "Sestre milosrdnice", Institut for tumors, Zagreb, Croatia

Corresponding author: Prof. Marko Samardzija, Faculty of Veterinary Medicine, University in Zagreb, Croatia, Heinzelova 55, 10000 Zagreb, Croatia, email: smarko@vef.hr

ABSTRACT

The aim of research was to evaluate intrauterine ozone therapy in Holstein cows with retained fetal membranes (RFM). Research is performed on 45 Holstein cows aged 2-6 years divided in three groups. The first group (n=15) consisted of cows treated with foam spray ozone application with sterile catheter in the body of the uterus for 5 seconds. The second group (n=15) was consisted of cows treated using 6 ozone perls. The third group or control group (n=15) was consisted of cows treated with foaming tablets (oxytetracyclin). Cows were observed and treated during early puerperium, 24 to 36 hours after parturition. To assess the reproductive performance of cows, interval from calving to first insemination (days open to first service, DOFS), interval from calving to pregnancy (days open to pregnancy, DOP), and all service conception rate (ASCR) were measured. Conception rate was 1.80 in the first group, similar to control group (1,73) and 2.07 in cows treated with ozone perls. In first group, days open to first service was 101.06 ± 16.29, in second 108.53 ± 16.09 and the third 104.53 ± 13.56 days. Days open to pregnancy was shortest in the first group (118.06 ± 24.85), but the longest in cows treated with ozone perls (133.20 ± 26.96) and 121.20 ± 32.32 days in cows treated with OTC foaming tablets. Cows with RFM treated with intrauterine Riger spray or Ripromed ovuli O₃ have similar reproductive performance results as in control group of cows which proves the effectiveness of therapy with intrauterine ozone products.

KEY WORDS: *Cow, Ozone, Retained Placenta*

INTRODUCTION

Uterine disorders are often causes of dairy cows infertility. They reduce the reproductive efficiency of cows (*Lewis* 1997) by extending the calving to conception interval, increasing the number of inseminations per conception (*Nakao et al.* 1992; *Gilbert et al.* 2005; *Kim and Kang* 2003) and by increasing the culling rates (*LeBlanc et al.* 2008; *Gilbert et al.* 2005). Most of diseases that may cause infertility, occur in peripartal period, such as metritis, endometritis (*Gautam et al.* 2009), retention of foetal membranes (RFM) and some metabolic disorders (*Paisley et al.* 1986, *Laven and Peters* 1996; *Levis* 1997; *Sheldon and Dobson* 2004; *Han and Kim*, 2005; *Könyves et al.* 2009). Incidence of uterine infection in postpartum dairy cows is 40 (10-50) % (*Lewis* 1997; *Noakes et al.* 2009). RFM delays uterine involution and could lead to endometritis and metritis resulting with subfertility (*Dinsmore et al.* 1996; *Gröhn and Rajala-Schultz* 2000; *Drillich et al.* 2003; *Kim and Kang* 2003; *Maizon et al.* 2004; *Gautam et al.* 2009). The fetal membrane is expelled 6 to 8 hours after calving in about 77% of cows (*Van Werven et al.* 1992). RFM in cows are defined as absence of foetal membrane expulsion within 12 to 24 hours after calving (*Fourichon et al.* 2000; *Noakes et al.* 2009). The incidence of RFM